Supplementary Figure legends

Figure S1. The expression of NEAT1 was upregulated in LPS-induced DC maturation. **(A)** The expression of NEAT1 in DCs stimulated with the agonists lipoteichoic acid (TLR2), polyinosinic-polycytidylic acid (TLR3), and TLR7/8 (R848) for 24 h was detected by qRT-PCR. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, ns, not significant, derived using Student's t-test. **(B)** DCs were stimulated with LPS (10 ng/ml, 200 ng/ml, or 1 μg/m). NEAT1 v1 expression was determined by qRT-PCR at different time points (0, 4, 8, 12, and 24 h). **(C-D)** DCs were treated with Lipofectamine or LPS for 12 h, and the expression levels of co-stimulators (CD80, CD86, and MHC II) were detected by flow cytometry. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, derived using Student's t-test. **(E)** DCs were treated with Lipofectamine or LPS for 12 h, and the expression of NEAT1v2 was detected by qRT-PCR. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, derived using Student's t-test.

Figure S2. Knockdown of NEAT1 induces tolerogenic DCs. **(A)** qRT-PCR was used to detect the expression of NEAT1 after siRNA-mediated knockdown of NEAT1 in DCs. **(B)** The expression levels of co-stimulators (CD80, CD86, and MHC II) were detected by flow cytometry in DCs treated with NEAT1 siRNA (siNEAT1) or negative control (NC) for 12 h before treatment with LPS (200 ng/ml). Data are expressed as mean ± SD; n = 4 biological replicates; ***p<0.001, derived using Student's t-test. **(C-D)** The levels of IL-1β and IL-6 were analyzed by ELISA in DC supernatants. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, ****p<0.0001 derived using Student's t-test.

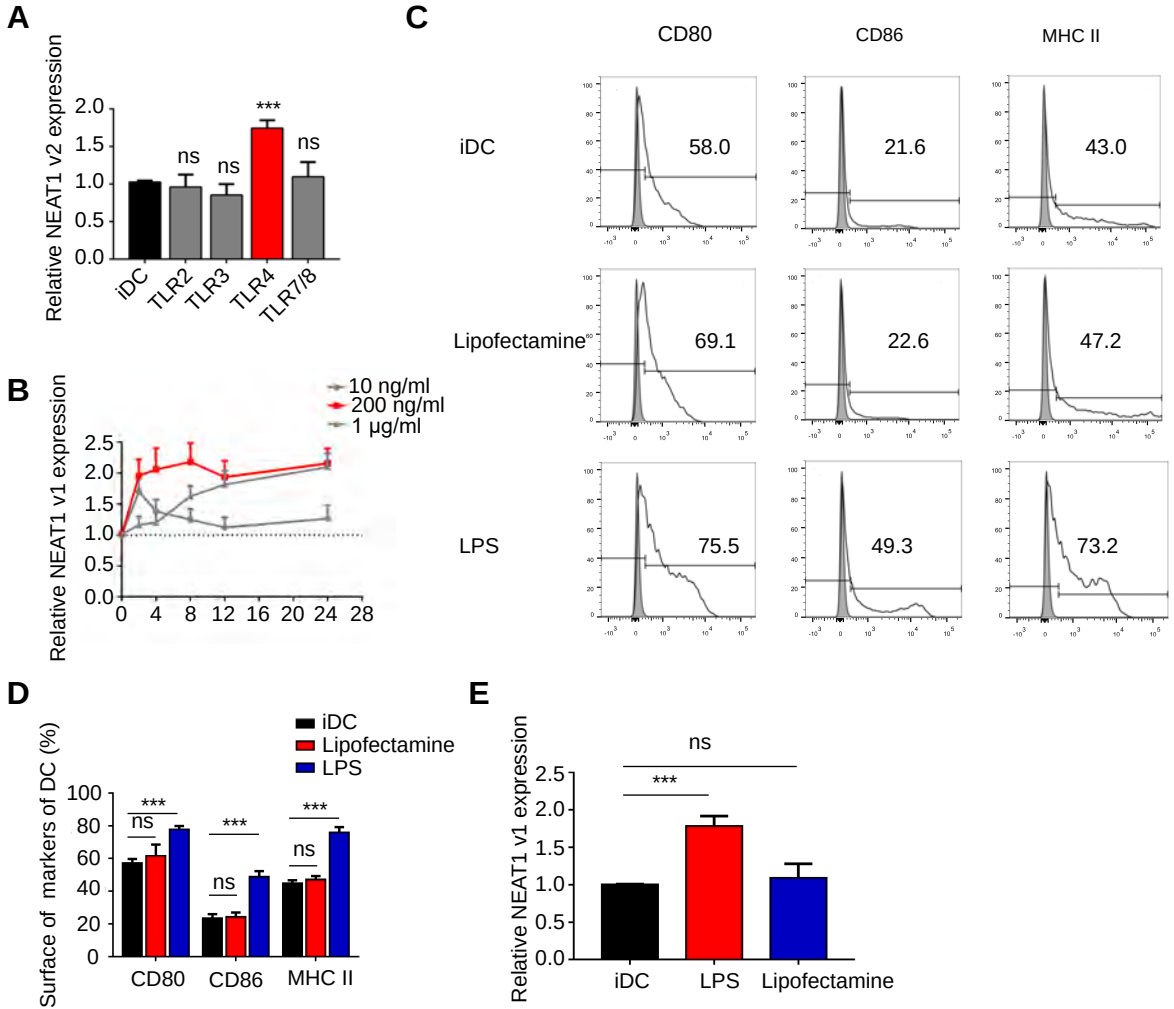
Figure S3. NEAT1 regulates the expression of NLRP3 inflammasome by competing for miR-3076-3p. **(A)** The altered miRNA levels were selectively confirmed by qRT-PCR in the knockdown of NEAT1. Data are expressed as mean ± SD; n = 3 biological replicates; **p<0.01, ***p<0.001, derived using Student's t-test. **(B)** DCs were treated with miR-3076-3p inhibitor or miR-3076-3p mimic for 12 h, and the expression of NEAT1v2 was detected by qRT-PCR. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, derived using Student's t-test. **(C-G)** DCs were transfected with miR-3076-3p mimic, miR-3076 inhibitor, or NC. Relative mRNA expression of caspase-1 (C) and ASC (D) was detected by qRT-PCR. The protein levels of NLRP3 (E), caspase-1 (F), and ASC (G) were detected by western blotting. Data are expressed as mean ± SD; n = 3 biological replicates; **p<0.01, ***p<0.001, ****p<0.0001, derived using Student's t-test. **(H-L)** DCs transfected with siNEAT1, DCs overexpressing NEAT1 (OE NEAT1), DCs treated with siNEAT1+miR-3076-3p inhibitor or OE NEAT1+miR-3076-3p mimic, and negative control DCs. Relative mRNA expression of caspase-1 (H) and ASC (I) was detected by qRT-PCR. The protein levels of NLRP3 (J), caspase-1 (K), and ASC (L) were detected by western blotting. Data are expressed as mean ± SD; n = 3 biological replicates; **p<0.01, ***p<0.001, ns, not significant, derived using Student's t-test.

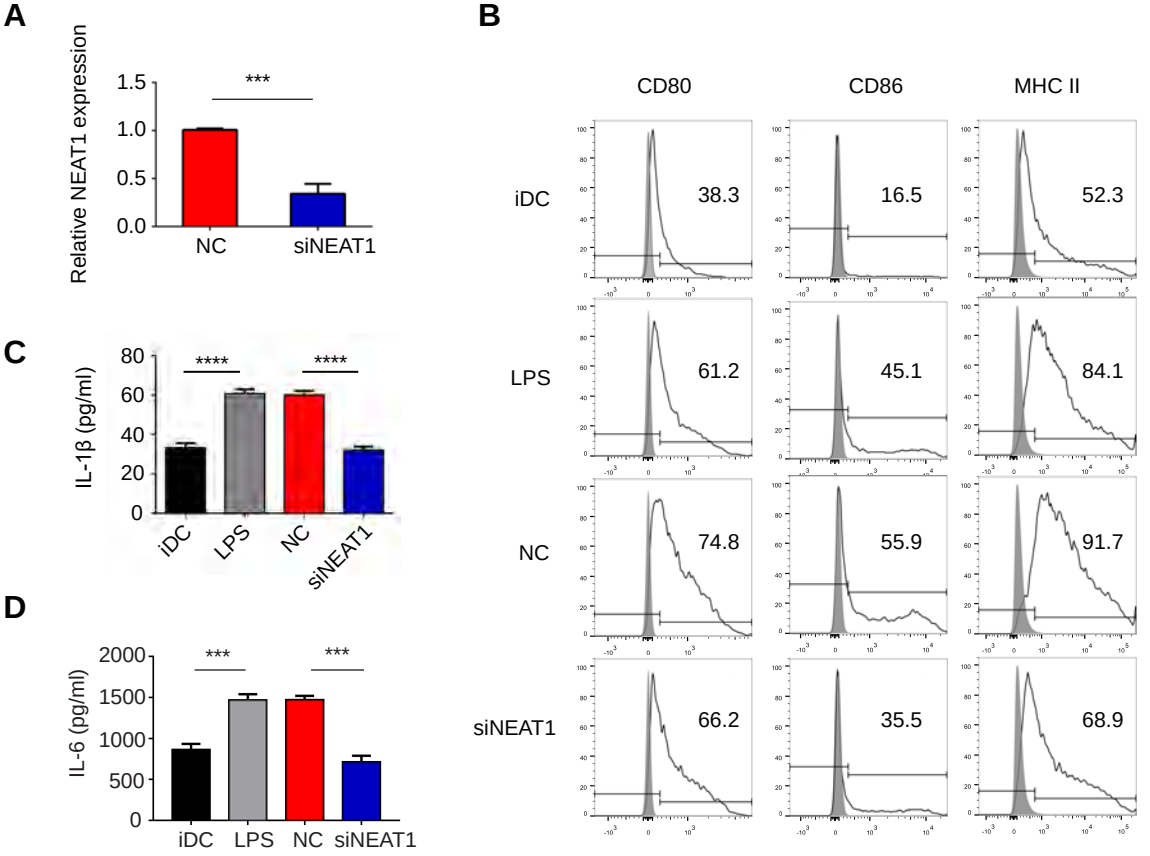
Figure S4. NEAT1 induces tol-DCs via miR-3076-3p/NLRP3 axis. **(A -B)** Western blotting was performed to detect the expression of NEAT1 after pcDNA-NLRP3 overexpression in DCs. Data are expressed as mean ± SD; n = 3 biological replicates; **p<0.01, derived using Student's t-test.

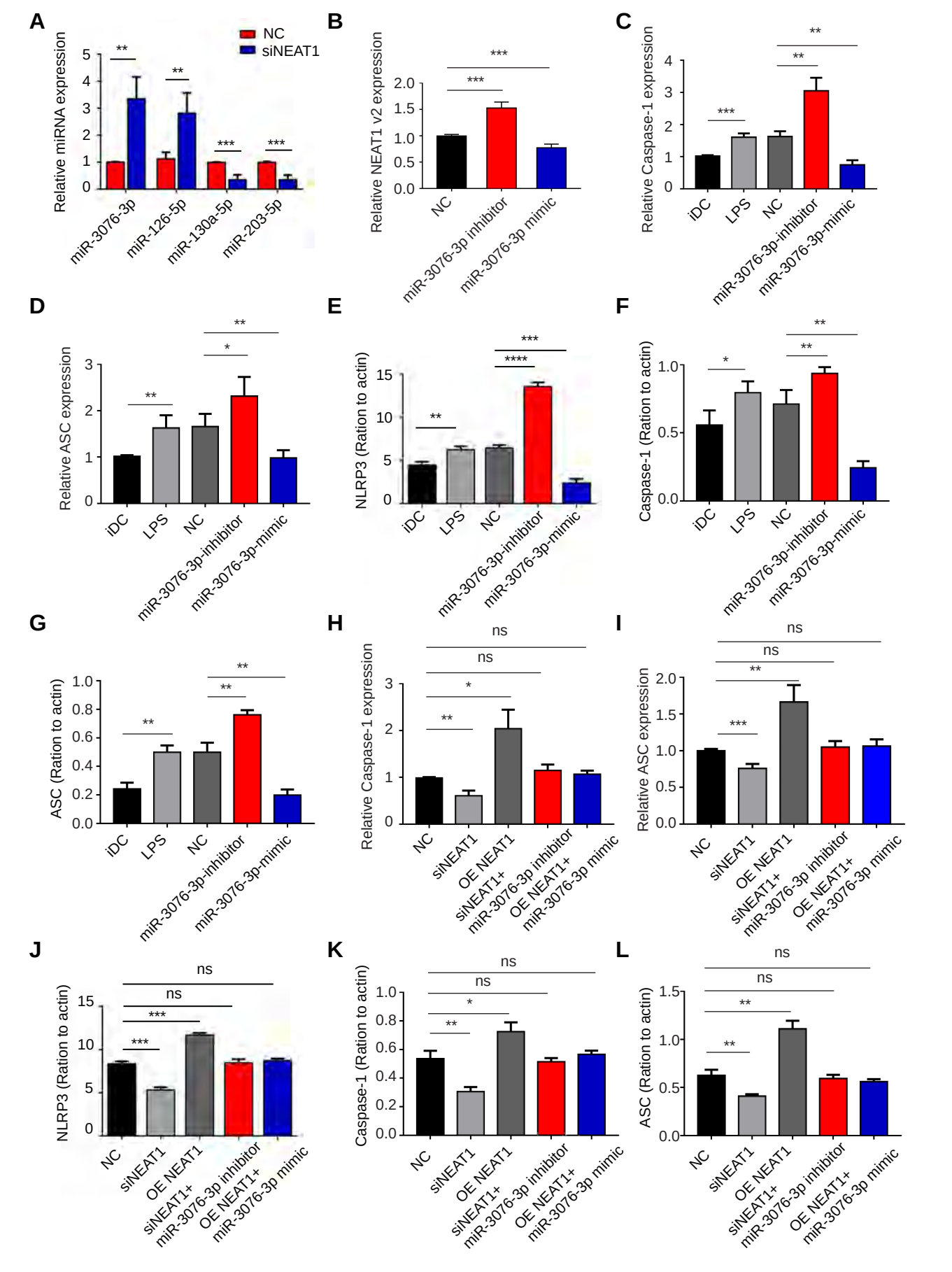
(C-D) DCs were treated with siNEAT1 or pcDNA-NLRP3, the control protein GAPDH was detected by western blot. Data are expressed as mean ± SD; n = 3 biological replicates; ns not significant, derived using Student's t-test. **(E)** The expression levels of co-stimulators (CD80, CD86, and MHC II) were detected by flow cytometry in DCs treated with siNEAT1, siNEAT1+pcDNA-NLRP3, or siNEAT1+pcDNA-NLRP3 in the presence of a neutralizing monoclonal antibody specific for IL-1β.

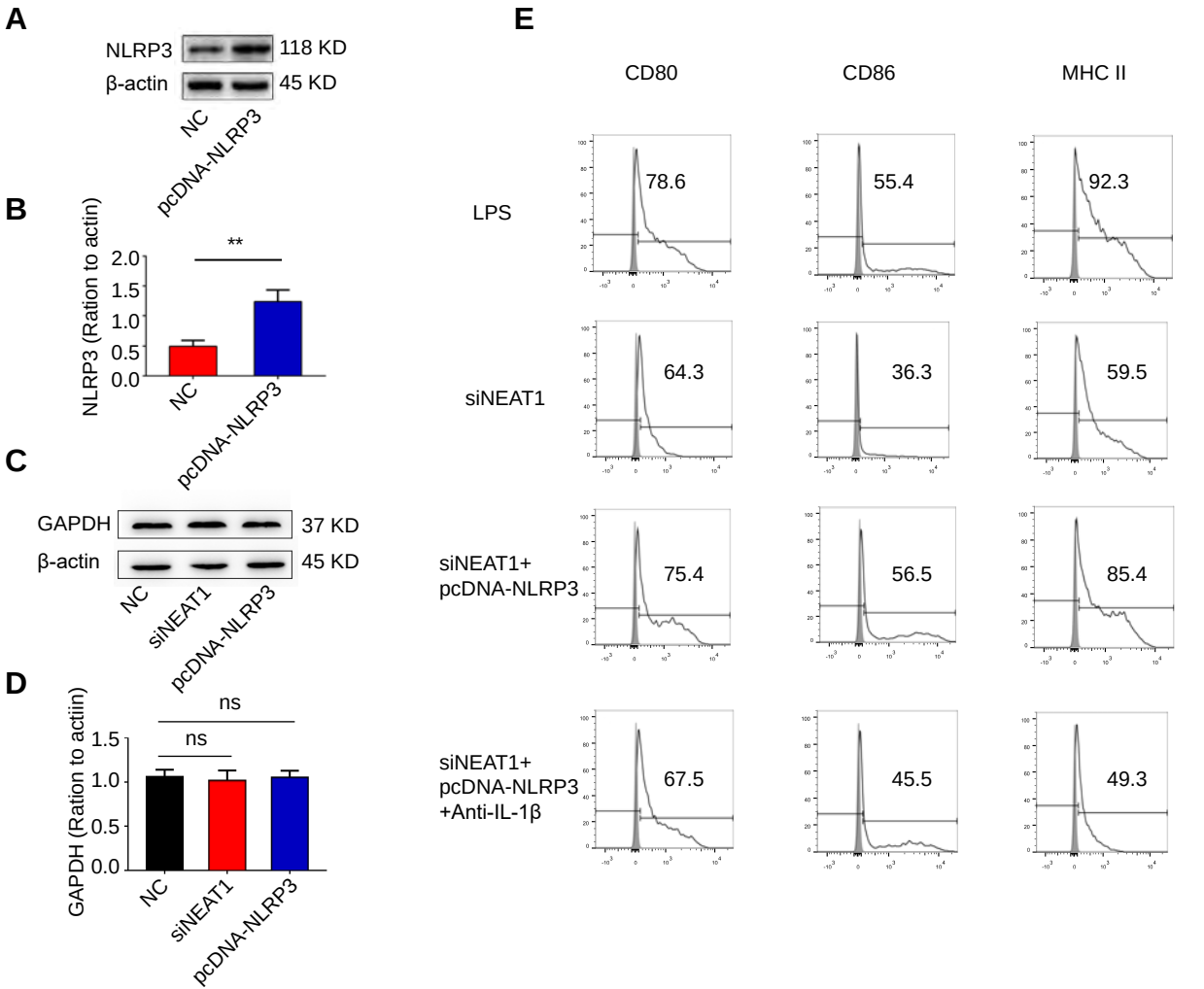
Figure S5. NEAT1 is regulated by the transcription factor E2F1. **(A)** The expression of E2F1 mRNA was detected by qRT-PCR in DCs treated with E2F1 siRNA, DCs overexpressing E2F1, or NC. Data are expressed as mean ± SD; n = 3 biological replicates; **p<0.01, ***p<0.001, ns, not significant, derived using Student's t-test. **(B-C)** The levels of the E2F1 protein were assessed by western blotting in DCs treated with E2F1 siRNA, DCs overexpressing E2F1, or NC. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, ns, not significant, derived using Student's t-test. **(D)** CpG island distribution in the promoter of NEAT1. Horizontal axis denotes the genome sequence of NEAT1. Vertical blue stripes indicate CpG islands. **(E)** DCs were transfected with siE2F1 or NC for 12 h before undergoing stimulation with LPS. Methylation status of the CpG island was assessed by bisulfite sequencing in immature DCs (iDCs), LPS-DCs, NC, and siE2F1 DCs. Each row represents a single clone. **(F)** DCs were stimulated with an inhibitor of DNA methyltransferase (5-aza-2'-deoxycytidine) used at different concentrations (0.5 μM, 0.05 μM, 0.01 μM, or 0.005 μM) for 12 h before undergoing stimulation with LPS. The expression of NEAT1 was detected by qRT-PCR. Data are expressed as mean ± SD; n = 3 biological replicates; ***p<0.001, ns, not significant, derived using Student's t-test.

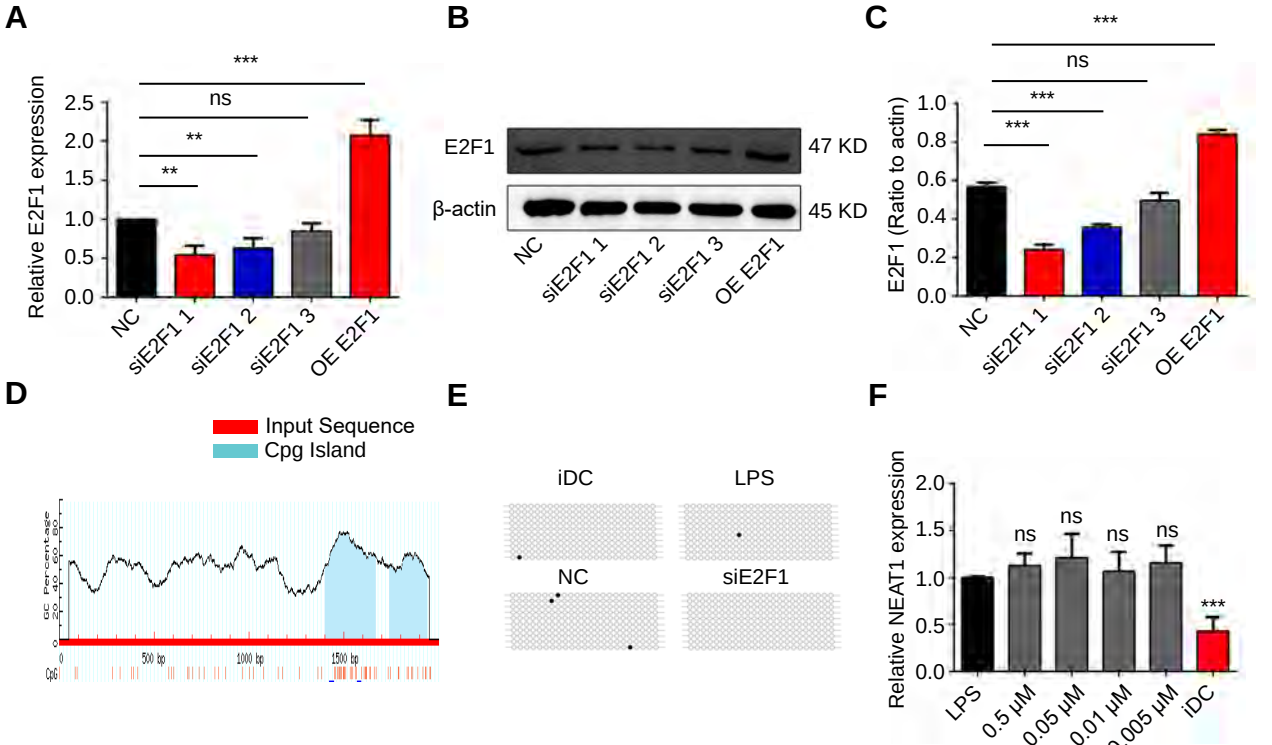
Figure S6. Expression of the components of the NLRP3 inflammasome in EAM mice subjected to infusion with NEAT1-knockdown DCs. **(A)** Hearts harvested from the PBS-injected group and the group transfected with NC-DCs were stained with hematoxylin and eosin (the PBS group was shown in Figure 5H). Scale bars correspond to 100 μm. **(B)** Assessment of hematoxylin and eosin staining by grading. Data are expressed as mean ± SD; n = 5 biological replicates; ns, not significant, derived using Student's t-test. **(C-E)** Immunohistochemical labeling for NLRP3 (C), caspase-1 (D), and ASC (E) in hearts harvested from normal, PBS-treated, and siNEAT1-treated groups. Scale bars correspond to 50 μm.


A
Relative NEAT1 v2 expression
2.0
1.5
1.0
0.5
0.0
ns
ns
***
ns
iDC
TLR2
TLR3
TLR4
TLR7/8
B
Relative NEAT1 v1 expression
10 ng/ml
200 ng/ml
1 µg/ml
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
28
C
CD80
CD86
MHC II
iDC
58.0
21.6
43.0
Lipofectamine
69.1
22.6
47.2
LPS
75.5
49.3
73.2
D
Surface of markers of DC (%)
iDC
Lipofectamine
LPS
100
80
60
40
20
0
***
ns
***
ns
***
ns
CD80
CD86
MHC II
E
Relative NEAT1 v1 expression
ns
***
2.5
2.0
1.5
1.0
0.5
0.0
iDC
LPS
Lipofectamine


A
Relative NEAT1 expression
1.5
1.0
0.5
0.0
***
NC
siNEAT1
B
CD80
CD86
MHC II
iDC
LPS
NC
siNEAT1
38.3
16.5
52.3
61.2
45.1
84.1
74.8
55.9
91.7
66.2
35.5
68.9
C
IL-1β (pg/ml)
80
60
40
20
0
****
****
iDC
LPS
NC
siNEAT1
D
IL-6 (pg/ml)
2000
1500
1000
500
0
***
***
iDC
LPS
NC
siNEAT1


A
NC
siNEAT1
Relative miRNA expression
miR-3076-3p
miR-126-5p
miR-130a-5p
miR-203-5p
B
Relative NEAT1 v2 expression
NC
miR-3076-3p inhibitor
miR-3076-3p mimic
C
Relative Caspase-1 expression
iDC
LPS
NC
miR-3076-3p-inhibitor
miR-3076-3p-mimic
D
Relative ASC expression
E
NLRP3 (Ration to actin)
F
Caspase-1 (Ration to actin)
G
ASC (Ration to actin)
H
Relative Caspase-1 expression
NC
siNEAT1
OE NEAT1
siNEAT1+ miR-3076-3p inhibitor
OE NEAT1+ miR-3076-3p mimic
I
Relative ASC expression
J
NLRP3 (Ration to actin)
K
Caspase-1 (Ration to actin)
L
ASC (Ration to actin)
ns


A
NLRP3
118 KD
β-actin
45 KD
NC
pcDNA-NLRP3
B
NLRP3 (Ration to actin)
2.0
1.5
1.0
0.5
0.0
**
NC
pcDNA-NLRP3
C
GAPDH
37 KD
β-actin
45 KD
NC
siNEAT1
pcDNA-NLRP3
D
GAPDH (Ration to actiin)
1.5
1.0
0.5
0.0
ns
ns
NC
siNEAT1
pcDNA-NLRP3
E
CD80
CD86
MHC II
LPS
78.6
55.4
92.3
siNEAT1
64.3
36.3
59.5
siNEAT1+
pcDNA-NLRP3
75.4
56.5
85.4
siNEAT1+
pcDNA-NLRP3
+Anti-IL-1β
67.5
45.5
49.3


A
Relative E2F1 expression
2.5
2.0
1.5
1.0
0.5
0.0
***
ns
**
**
NC
siE2F1 1
siE2F1 2
siE2F1 3
OE E2F1
B
E2F1
47 KD
β-actin
45 KD
NC
siE2F1 1
siE2F1 2
siE2F1 3
OE E2F1
C
E2F1 (Ratio to actin)
1.0
0.8
0.6
0.4
0.2
0.0
***
ns
***
***
NC
siE2F1 1
siE2F1 2
siE2F1 3
OE E2F1
D
Input Sequence
Cpg Island
GC Percentage
0
500 bp
1000 bp
1500 bp
Cpg
E
iDC
LPS
NC
siE2F1
F
Relative NEAT1 expression
2.0
1.5
1.0
0.5
0.0
ns
ns
ns
ns
***
LPS
0.5 μM
0.05 μM
0.01 μM
0.005 μM
iDC

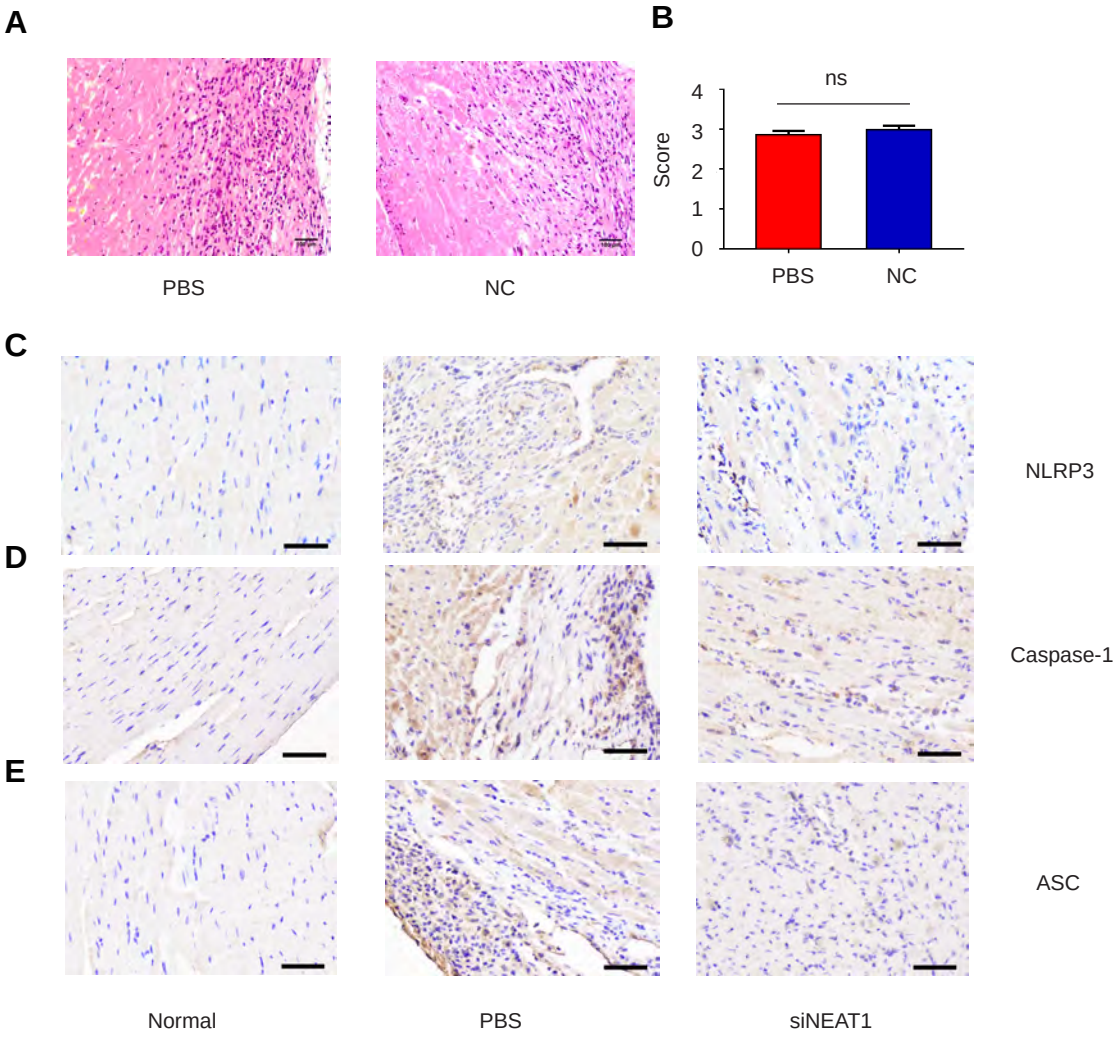

A
B
ns
Score
4
3
2
1
0
PBS
NC
PBS
NC
C
D
E
NLRP3
Caspase-1
ASC
Normal
PBS
siNEAT1

Table S2 Target sequence of NEAT1 Smart Silencer

Name	Target sequence of Smart Silencer
NEAT1 Smart Silencer	GCGCAAGTTAGCCACAAAT
	GAGGGATTCTTAACTGAAA
	GGAGGAATCTTCCTTAGAT
	TAACAGTGGGAAGGCGCAAG
	AGTCGGTGCTGGAGTCTTGG
	CCAGTGTGAGTCGTAGCAGT

Table S3 The list of PCR primer sequences and siRNA sequences

Primer name	Sequence 5'---3'
NEAT1_1 FO	TCCTCTACAGCCTTACCTACATC
NEAT1_1 RE	AGACAACCTTCAACCAACAACC
NEAT1_2 FO	CTTGTTCTGGGAGCATCAT
NEAT1_2 RE	CTACACCTTACGCAATCTTCT
U6 FO	ATTGGAACGATACAGAGAAGATT
U6 RE	GGAACGCTTCACGAATTTG
GAPDH FO	CCTTCATTGACCTCAACTACATGG
GAPDH RE	CTCGCTCCTGGAAGATGGTG
ACTB FO	CTTCTTTGCAGCTCCTTCGT
ACTB RE	CTTCTGACCCATTCCCACC
miR-3076-3p FO	GGCAAACGCACTCTGGTCTTC
miR-3076-3p RE	TATGGTTGTTCACGACTCCTTAC
NLRP3 FO	AGATGCTGGAATTAGACAACTG
NLRP3 RE	CATTTCACCCAACTGTAGGC
Caspase-1 FO	ACCACTCGTACACGTCTTGC
Caspase-2 RE	TGGGCAGGCAGCAAATTCTT
ASC FO	GAAGCTGCTGACAGTGCAAC
ASC RE	TGTGAGCTCCAAGCCATACG
E2F1 FO	TCACGCTATGAAACCTCACTAA
E2F1 RE	TTCAAGCCGCTTACCAATC
miR-126-5p FO	CTGCTCACATTATTACTTTTGGT
miR-126-5p RE	TATGGTTGTTCTGCTCTCTGTCTC
miR-130a-5p FO	GCCTTCACATTGTGCTACTGTCTGC
miR-130a-5p RE	CCAGTCTCAGGGTCCGAGGTATTC
miR-203-5p FO	GTGAGTATGTGGAGTAGTTTTTGG
miR-203-5p RE	ACACCTTTTATACATCTAAATCACCC
miR-let-7i inhibitor	AACAGCACAAACUACUACCUCA
microRNA inhibitor NC	CAGUACUUUUGUGUAGUACAA
miR-let-7i mimic sense	UGAGGUAGUAGUUUGUGCUGUU
miR-let-7i mimic antisense	CAGCACAAACUACUACCUCAUU
microRNA mimic NC sense	UUCUCCGAACGUGUCACGUTT
microRNA mimic NC antisense	ACGUGACACGUUCGGAGAATT
NEAT1 ChIP E2F1 FO	CCACCCAAGCTGCTGGG
NEAT1 ChIP E2F1 RE	GGGATCTCTGCGGTGGC
NEAT1 ChIP H3K27ac FO	CTAAGGCCTCCTCACCTCTC
NEAT1 ChIP H3K27ac RE	GGTCGCCTGGTCACGCACAG
NEAT1 RIP FO	CTTGCCACACCTTGTCTTGC
NEAT1 RIP RE	TAGCTGGTGCATCCTGTGTG
miR-3076-3p RIP FO	GGCAAACGCACTCTGGTCTTC
miR-3076-3p RIP RE	TATGGTTGTTCACGACTCCTTAC
NEAT1 CpG1 FO	TTTATTTTGTTGTTGTTYGTATTGA
NEAT1 CpG1 RE	TCACACCRAATATCAACRCTAAA

NEAT1 CpG2 FO	TTATTTTYGGTGGTGTTTATYGT
NEAT1 CpG2 RE	TCACCCAAACACTACTAAACRAAAC
siE2F1-1 sense	GUGGAUUCUUCAGAGACAUTT
siE2F1-1 antisense	AUGUCUCUGAAGAAUCCACTT
siE2F1-2 sense	CGCUAUGAAACCUCACUAATT
siE2F1-2 antisense	UUAGUGAGGUUUCAUAGCGTT
siE2F1-3 sense	GAGGGCAUUAGAGAUCUCUTT
siE2F1-3 antisense	AGAGAUCUCUAAUGCCCUCTT